International Journal of Pharmacy and Chemistry
2024; Vol. 10, No. 1, pp. 1-9
https://doi.org/10.11648/j.ijpc.20241001.11

Research Article

Phytotherapeutic Effects of Ashanti Pepper and Horn of Plenty in Sleep Disorder Management Resulting from Stress-Induced Neurobehavioral Alterations

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Abstract

This investigation explores the potential antidepressant effects arising from the inherent bioactive compounds found in *Cnestis ferruginea* and *Piper guineense*. Both botanical entities contain flavonoids, phenolics, saponins, and tannins, which are known for their anxiolytic, antidepressant, and antioxidant properties. It is worth noting that *Cnestis ferruginea* also contains terpenoids, which are recognized for their anxiolytic effects. Given the growing interest in nutritional interventions for neuropsychiatric conditions, our study meticulously examines the antioxidant capacities and anxiolytic properties of these phytochemicals, positioning them as potential regulators of brain health, particularly in the realm of sleep disorders. In response to the increasing demand for therapeutic alternatives with reduced side effects, our inquiry thoroughly examines the antioxidant and anxiolytic profiles of these botanical extracts. Of particular importance, *Cnestis ferruginea* exhibits superior antioxidant potential compared to *Piper guineense*, thus warranting further investigation into its pharmacological utility. Subsequent analysis of the effects of *Cnestis ferruginea* on sleep architecture, especially in the context of chronic stress, reveals promising results. The botanical extract significantly prolongs sleep duration in rats exposed to stress, suggesting a potential dual role in mitigating stress-induced neurobehavioral changes and depressive symptoms. Additionally, a noticeable decrease in immobility duration in the forced swim test, along with improvements in acetylcholinesterase levels following *Cnestis ferruginea* treatment, provides mechanistic insights into its antidepressant effects. Our findings provide substantial evidence to support the pharmacotherapeutic potential of *Cnestis ferruginea*, highlighted by its antioxidant abilities and anxiolytic effects. Further investigations should delve into the intricate molecular pathways underlying these effects, potentially paving the way for innovative botanical-based interventions in neuropsychiatric disorders, particularly those associated with stress-induced neurobehavioral changes and sleep disorders.

Keywords

Cnestis ferruginea, *Piper guineense*, Antidepressant Effects, Anxiolytic Properties, Antioxidant Potentials, Sedative Activity, Nutritional Interventions, Sleep Disorders

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Received: 6 December 2023; **Accepted:** 6 January 2024; **Published:** 2 April 2024

1. Introduction

Understanding the intricate effects of stress on human behavior and sleep remains a persistent challenge in the field of health. This research delves into the potential of natural remedies, such as Horn of Plenty (*Cnestis ferruginea*), Ashanti pepper (*piper guineense*), in mitigating these impacts. By exploring the ability of these botanical interventions to modulate the influence of stress on neurobehavioral responses and sleep patterns, we aim to unveil a more holistic approach to managing sleep disorders.

The stress response is orchestrated by the hypothalamus in the brain, prompting the release of signaling molecules that activate the pituitary and adrenal glands. This cascade stimulates the secretion of hormones like cortisol and adrenaline, crucial for the body's reaction to stressors [1]. These hormones elicit a spectrum of biochemical changes, increasing heart rate, blood pressure, and respiration rates, while simultaneously suppressing digestive and immune functions [2]. Despite acute stress offering potential benefits in certain circumstances, chronic stress exerts a profound biochemical toll on mental and physical health.

Chronic stress has been linked to numerous health problems, including depression, anxiety, heart disease, and diabetes. Symptoms of stress can vary from person to person, but some common physical symptoms include headaches, muscle tension, fatigue, and sleep disturbances. Emotional symptoms of stress can include anxiety, irritability, mood swings, and difficulty concentrating. Long-term stress can also lead to changes in behavior, such as social withdrawal, substance abuse, and overeating. Managing stress is important for maintaining overall health and wellbeing.

Ashanti pepper, a plant commonly found in West Africa, is renowned for its culinary and medicinal properties and has historically been used to alleviate conditions such as fever, malaria, and pain. Within the Ashanti pepper, there exists a bioactive compound known as piperine, which has demonstrated potent antioxidant and anti-inflammatory characteristics. In the domain of chronic stress, the utilization of the plant extract has the potential to alleviate stress-induced neurobehavioral changes by counteracting both oxidative stress and inflammation, both of which have been identified as factors contributing to neuronal damage and alterations in behavior. The extract's ability to scavenge free radicals and reduce oxidative stress within the brain holds promise for the protection of neural tissues. Additionally, the extract may possess the ability to modulate immune response and decrease brain inflammation, potentially contributing to the amelioration of stress-induced behavioral changes.

Horn of plenty, another plant indigenous to West Africa, has a long-established history of traditional medicinal use for various conditions. Recent studies have focused on its impact on stress-induced neurobehavioral alterations and cognitive function, suggesting a potential role in enhancing cognitive abilities. Research, such as the study conducted by [3], has demonstrated significant enhancement in cognitive function in rats subjected to chronic unpredictable stress. While the specific mechanisms by which the extract of *Cnestis ferruginea* influences stress-induced neurobehavioral changes have yet to be fully elucidated, this investigation aims to examine the modulatory effects of both Ashanti pepper and Horn of plenty on stress-induced neurobehavioral changes in male albino Wistar rats.

2. Methods

2.1. Preparation of Plant Extract

The seeds underwent a process of washing, in which they were cleansed and then subsequently reduced in size to promote an increase in the overall surface area. Following this, the seeds were left to dry at ambient temperature, after which they were pulverized utilizing a mechanical blender. The resultant pulverized sample, weighing 25 grams, was subjected to maceration in a solution consisting of 80% ethanol, during which intermittent shaking occurred. Following a duration of 24 hours, the resulting mixture was subjected to filtration and subsequently utilized in a Rotary Evaporation process, resulting in the extraction of a desired compound. The weight of the extracted compound was measured in order to determine the percentage yield, and it was subsequently stored for utilization in both in vitro and in vivo experiments.

2.2. Preliminary Phytochemical Screening

Phytochemical screening of the extract was carried out using standard phytochemical methods. The screening involves the detection of phenols, tannins, alkaloids, flavonoids, terpenoids, anthraquinones, saponins.

2.3. Quantitative Phytochemical Screening

2.3.1. Total Phenolic

The total phenolic content of extract was determined using the Folin-Ciocalteu's method of Waterman and Mole [4].

Principle: In this assay, the phenolic group present in the extract interacts with Folin - Ciocalteau's in alkaline medium using Na_2CO_3 solution giving a blue colour, which has maximum absorption at 760 nm and correlates with total phenolic content.

Procedure: Exactly 0.1 ml of extract (1 mg/ml) was rapidly mixed with 0.1 ml of Folin Ciocalteu reagent, followed by the addition of 0.1 ml sodium carbonate (20%, w/v) solution. The mixture was incubated in the dark for 30 min. The absorbance of the blue colour was read at 760 nm after 60 min on a spectrophotometer. The total phenolic content was extrapolated from a standard curve using tannic acid or gallic acid (graded concentra-

tion, 50-250 µg/ml) as a standard. The amount of total phenolics was expressed as Tannic acid equivalent (TAE, mg tannic acid/g sample) through the calibration curve of Tannic acid.

2.3.2. Total Flavonoid

The total flavonoid content was determined spectrophotometrically according to the modified method of Kumaran and Karunakaran [5].

Principle: Aluminium chloride forms stable complexes with C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols. Aluminium chloride also forms acid labile complexes with the ortho-dihydroxyl groups in the A- or B-ring of flavonoids.

Procedure: Briefly, 0.5 ml of extract solution and standard (quercetin) at different concentrations (12.5-200 µg/ml) were taken in test tubes dissolved in ethanol followed by the addition of 0.1 ml of 10% aluminum chloride solution. 0.1 ml of 1 M potassium acetate solution was added to the mixtures in the test tubes. Furthermore, each reaction test tube was then immediately diluted with 0.8 ml of distilled water and mixed to incubate for 30 min at room temperature to complete reaction. The absorbance of pink colored solution was noted at 415 nm using a spectrophotometer against blank methanol. Total Flavonoid Concentration (TFC) of the extract was expressed as quercetin equivalents (QE).

2.3.3. Determination of Total Tannin

They were determined by Folin-Ciocalteu method as described by Broadhurst [6].

Procedure: The extract (0.1 ml) was added to a flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu phenol reagent, I ml of 35% Na_2CO_3 solution and dilute to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of Tannic Acid (0.2, 0.4, 0.6, 0.8, 1 mg/ml) were prepared. Absorbance for test and standard solutions were measured against the blank at 725 nm with an UV/Visible spectrophotometer. The tannin content was expressed in terms of mg of CE /g of extract.

Evaluation of the Antioxidant Activities.

2.3.4. Determination of ABTS Radical Scavenging Activity

ABTS radical scavenging activity of the tea samples was determined using ABTS antiradical assay as described by Awika [7].

Principle: 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS") decolourization test is a spectrophotometric method widely used for the assessment of antioxidant activity of various substances. The method is based on scavenging of the long-lived radical, ABTS (generated by oxidation of ABTS with potassium persulfate) by potential antioxidants. Trolox is used as reference standard and the antioxidant properties of these substances are expressed as trolox equivalent antioxidant capacity (TEAC).

Procedure: The ABTS" (mother solution) was prepared by mixing equal volumes of 8 mM ABTS and 3 mM potassium persulphate (K_2S_2O) (both prepared using distilled water) in a volumetric flask, which was wrapped with aluminum foil paper and allowed to react for a minimum of 12 h in a dark place. The working solution was prepared by mixing 5 mL of the mother solution with 145 mL phosphate buffer (pH 7.4). A range of trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman- carboxylic acid) standard solutions (100-1000 µM) were prepared in acidified methanol. The working solution (2.9 mL) was added to the tea samples (0.1 mL) or Trolox standard (0.1 mL) in a test tube and mixed with a vortex. The test tubes were allowed to stand for exactly 30 min. The absorbance of the standards and samples were measured at 734 nm with a Lambda EZ150 spectrophotometer. The result was expressed as µmol Trolox equivalents/g sample, on dry weight basis.

2.3.5. Determination of DPPH Radical Scavenging Activity

DPPH radical scavenging activity was done according to the method of Brand-Williams [8], with some modifications.

Principle: The relatively stable 2,2-dipheny-1-picrylhydrazyl (DPPH) free radical is used for antioxidant activity measurement of lipid soluble compounds. It is known that a freshly prepared DPPH. Solution exhibits a deep purple color with an absorption maximum at 517 nm. This purple color generally fades/disappears when an antioxidant molecule can quench DPPH. (By providing hydrogen atoms or by electron donation, conceivably via a free radical attack on the DPPH. molecule) and convert them to a bleached product (i.e., 2,2-diphenyl-1-hydrazine, or a substituted analogous hydrazine), resulting in a decrease in absorbance.

Procedure: The stock solution was prepared by dissolving 24 mg DPPH with 100 ml. methanol and then stored at -20 ℃ until needed. The working DPPH solution was obtained by mixing 10 ml. stock solution with 45 mL. methanol to obtain an absorbance of 1.1 units at 517 nm using the spectrophotometer. 0.5 mL of the tea samples was diluted with 2 mL of methanol to obtain a mother solution. 150 µL of the mother solution were allowed to react with 2850 µL of the DPPH working solution for 6 h in the dark.

2.4. Animal Grouping for Sedative Test

In this study, 21 rats were randomly grouped into seven groups based on the concentration difference (i.e 50 and 100 mg of the extract).

2.5. Diazepam Induced Sleeping Time Test

In the diazepam induced sleeping time test, two groups of three animals each were taken for each sample and another group of three animals as control. The control group received diazepam injection and other test group received appropriate

extract. The extract was administered orally with their respective doses. After 30 minutes of administration of respective extract, both the groups were administered with diazepam (1.5 Mg/Kg body weight) intraperitoneally. Rats were observed for loss of righting reflex (normal posture of the rat) and it was considered as the sign of onset of sleep. The interval between loss and recovery of righting reflex was used as the index of hypnotic activity in the test samples.

2.6. Animal Grouping for Antidepressant Studies

The rats were divided into 7 groups of three animals each. Group 2 to 5 are subjected to stressors listed above while Group 1, 6 and 7 are not stressed. This study was carried out for 14 days [9].

2.7. Neurobehavioral Assessments

2.7.1. Open-Field Test

A modified method of Abdelsalam and Safar [10] was employed for this assessment. The open- field was carried out using a square wooden box measuring 80 x 80 x 40 cm with red walls and white smooth polished floor divided by black lines into 16 equal squares 4 x 4. Each rat was placed gently in the central area of the open-field and allowed to freely explore the area for 5 min. The flour and walls were cleaned with 70% ethanol after testing each rat to eliminate possible bias because of odors left by previous rats. The number of squares crossed, hopping, rearing and number of fecal discharges within 300 s were recorded. Locomotor Activity was calculated as numbers of square crossed per movement stops.

2.7.2. Tail Suspension Test

A modified method of Steru [11] was employed for this assessment. Each mouse was suspended for 6 min by the tail (2 cm from the end of the tail) using adhesive tape. After the first 2 min of the test, total duration of immobility (in seconds) was measured. An animal was judged to be immobile when it ceased moving limbs and body, making only movements allowing to breathe.

2.7.3. Forced Swim Test (FST)

FST was carried out according to the method of Porsolt [12]. Each mouse was placed individually for 6 min into a glass cylinder (height 25 cm, diameter 10 cm) with 15 cm of water at 23–25 ℃. After the first 2 min of the test, total duration of immobility (in seconds) was measured. An animal was judged to be immobile when it ceased struggling and remained floating motionless and making only movements allowing to keep the head just above the surface of water.

2.8. Collection of Brain for Biochemical Estimations

Twenty-four hours after the last behavioral test, the rats were sacrificed by cervical dislocation and the brains of the sacrificed rats were excised and washed in ice cold 1.15% (v/v) potassium chloride solution, blotted with filter paper and weighed. They were then homogenized in 10% (w/v) phosphate buffered saline PBS (pH 7.4) using a Teflon homogenizer. The resulting homogenate was centrifuged at 10,000 x g at 4 ℃ for 25 minutes to obtain the supernatant which was used for biochemical analyses.

2.9. Evaluation of Acetylcholinesterase (AChE) Activity

Acetylcholinesterase (AChE) activity was measured by the spectrophotometric method developed by Ellman [13].

Principle

The principle of the method is the measurement of the rate of production of thiocholine as acetylthiocholine is hydrolyzed.

Procedure

0.1mL of 0.01 M DTNB was added to 2.6 mL of 0.1 M phosphate buffer (pH 8.0), 0.04 mL of brain homogenate was added to the above mixture followed by incubation for 5 minutes, after incubation, 0.04 mL of substrate (0.075 M acetylcholine iodide) was added to the reaction mixture. Absorbance readings were taken at 420 nm continuously for 3 minutes at 30 seconds intervals.

2.10. Evaluation of Butyrylcholinesterase (BuChE) Activity

Butyrylcholinesterase activity was measured by the spectrophotometric method developed by Ellman [13].

Procedure

0.1mL of 0.01 M DTNB was added to 2.6 mL of 0.1 M phosphate buffer (pH 8.0), 0.04 mL of brain homogenate was added to the above mixture followed by incubation for 5 minutes, after incubation, 0.04 mL of substrate (0.075 M butyrylcholine iodide) was added to the reaction mixture. Absorbance readings were taken at 420 nm continuously for 3 minutes at 30 seconds intervals.

3. Results

3.1. Phytochemical Screening

The Ethanolic of the sample *Cnestis ferruginea* and *Piper guineense* was screened for the presence of some phytochemicals. The sample was screened with 11 phytochemical tests and the sample tested positive to the presence of 8 phytochemicals. The result is shown in table 1.

Table 1. Qualitative phytochemical screening of ethanolic extracts of Cnestis ferruginea.

Sample	Flavanoids	Phenolics	Saponins	Tannins	Anthraquinones	Terpenoids
Cnestis ferruginea	+	+	+	+	-	-
Piper guineense	+	-	+	+	-	-

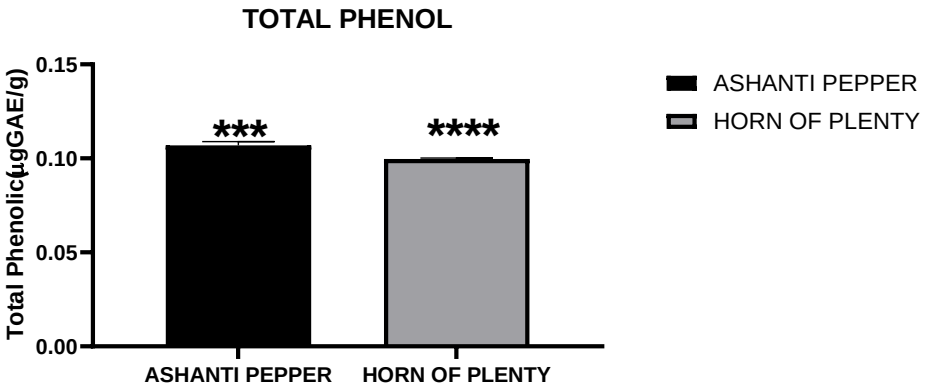
+ = present, ++ = abundant, +++= very abundant, ++++= very much abundant, - = absent

3.2. In-Vitro Antioxidant Evaluation

The ethanolic extract was screened against 7 antioxidant assays, and the results are shown below.

3.2.1. Total Phenolic Content

Values represent mean ± standard deviation of replicate experiments (n = 3). Values are significantly different (P< 0.05).

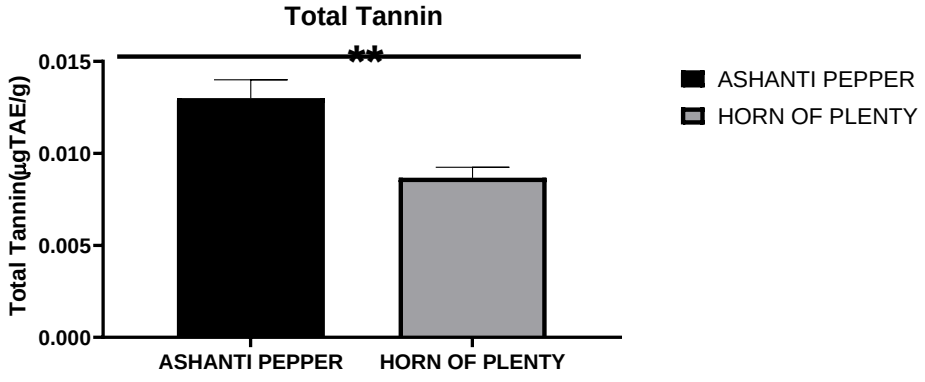


GAE = Gallic Acid Equivalent

Figure 1. Total Phenol content evaluation.

3.2.2. Total Tannin Content

Values represent mean ± standard deviation of replicate experiments (n = 3). Values are significantly different (P< 0.05).

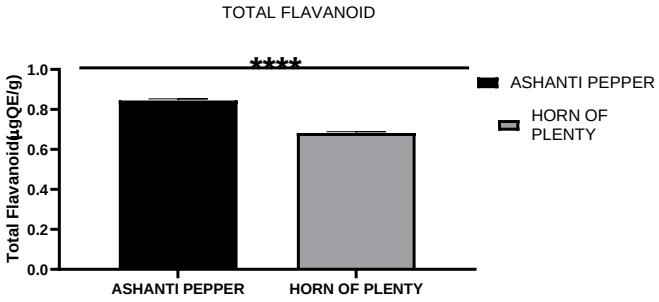


TAE = Tannic Acid Equivalent

Figure 2. Total tannin content evaluation.

3.2.3. Total Flavonoid Content

Values represent mean ± standard deviation of replicate experiments (n = 3). Values are significantly different (P< 0.05).



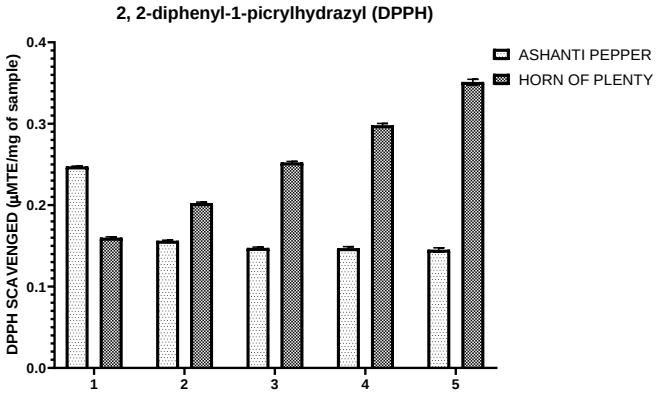
QE = Quercetin Equivalent

Figure 3. *Total flavonoid content determination.*

3.3. Radical Scavenging Ability of Extracts

3.3.1. 2,2-diphenyl-1-picrylhydrazyl (DPPH)

The scavenging ability of sample extracts, expressed in Trolox equivalent as shown.

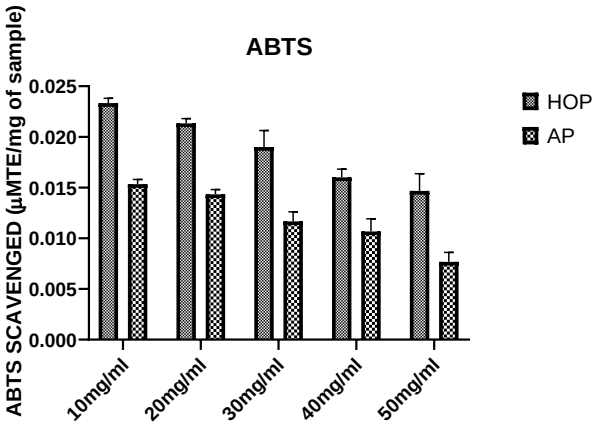


Values represent mean ± standard deviation of replicate experiments (n = 3). Values are significantly different (P< 0.05)

Figure 4. *2, 2-diphenyl-1-picrylhydrazyl (DPPH).*

3.3.2. 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS)

The scavenging activity was expressed in µM of Trolox equivalent as shown.



Values represent mean ± standard deviation of replicate experiments (n = 3). Values are significantly different (P< 0.05)

Figure 5. *ABTS radical scavenging ability extract.*

3.4. Sleep Potentiating Effect of *Cnestis ferruginea* Ethanolic Extract on Diazepam-Induced Sleeping Time in Male Wistar Rats

Table 2. *Sleep Potentiating Effect of Cnestis ferruginea Ethanolic Extract on Diazepam-Induced Sleeping Time in Male Wistar Rats.*

Grouping	Sleeping time (Mins)
Control	271 ±34secs
Stress	255 ±53secs
Stress + fluoxetine	267 ±47sec
Stress + Sample 250mg	283 ±32secs
Stress + Sample 500mg	268 ±4secs
Sample 250mg	298 ±26secs
Sample 500mg	278 ±20secs

Values represent mean ±standard deviation of replicate experiments (n = 3). Values are significantly different (P< 0.05).

3.5. Neurobehavioral Evaluation of the Effects of *Cnestis ferruginea* Ethanolic Extract on Stress-Induced Depressive Rats

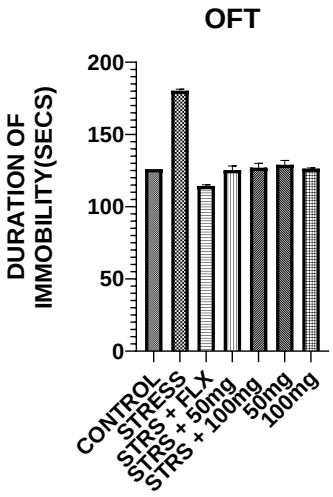


Figure 6. *Duration of immobility in open field test in stress-induced depressive rats.*

The stress only group showed a greater degree of despair depicted by their greater duration of immobility in water compared to the control group. Administration of both doses of *Cnestis ferruginea* ethanolic extract greatly reduced the duration of immobility of the tested rats (p<0.0001).

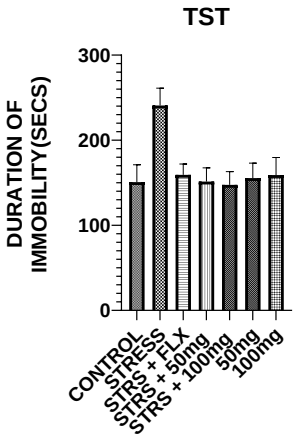


Figure 7. *Duration of immobility in Tail suspension test in stress-induced depressive rats.*

Figure 7 shows the effect of the two doses of *Cnestis ferruginea* ethanolic extract on forced swim test in stress-induced depressive rats. The depressed group was observed to show greater duration of immobility when suspended compared with the control group.

3.6. Effects of Extract Administration on the Level of Neurotransmitters in the Brain of Stress-Induced Depressive Rats

3.6.1. Acetylcholinesterase Level

The acetylcholinesterase level in the brain of rats treated with the ethanolic extract in Stress-induced depression. The depressed group (Stress) shows a significant reduction in the level of the neurotransmitter in their brain when compared with the control group (p<0.0001). This condition was improved by the administration of the extract in both dose concentrations 250 mg/kg/bw and 500 mg/kg/bw (p<0.0001).

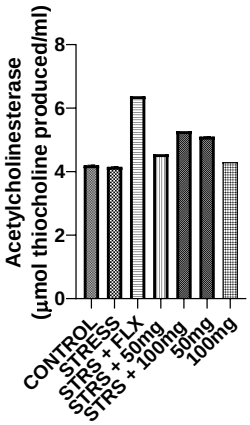


Figure 8. *Effect of Cnestis ferruginea extract on acetylcholinesterase activity in the brain of stress-induced depressive rats.*

3.6.2. Butyrylcholinesterase Level

The butyrylcholinesterase level in the brain of rats treated with the ethanolic extract in stress-induced depression. There was a significant decrease in the level of butyrylcholinesterase in the brain of the depressed (Stress). The depressed group (stress) showed a significant reduction in the level of the butyrylcholinesterase in their brain when compared with the control group ($p<0.0001$). This condition was improved by the administration of the extract ($p<0.0001$).

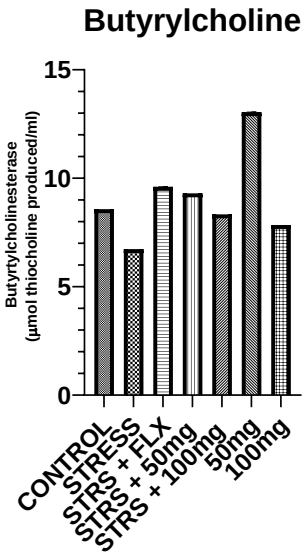


Figure 9. *Effect of Cnestis ferruginea extract on butyrylcholinesterase activity in the brain of stress-induced depressive rats.*

4. Discussion

The examination of *Cnestis ferruginea* and *Piper guineense* led to the discovery of a range of bioactive compounds that may contribute to their antidepressant effects. Both plants contain flavonoids, phenolics, saponins, and tannins, all of which have been identified for their anxiolytic, antidepressant-like, and antioxidant properties in animal studies [9]. Although anthraquinones were not found, *Cnestis ferruginea* exhibited the presence of terpenoids, which are acknowledged for their potential in reducing anxiety and promoting antidepressant effects.

In light of the growing emphasis on nutritional interventions, particularly in the treatment of depression, this study examines the antioxidant capabilities and sedative qualities of these plants [14]. The nutritional compositions of these plants make them potential contributors to enhancing brain health, an essential aspect in addressing sleep disorders. The utilization of nutritional strategies as a means of treating various conditions, including sleep disorders, has gained prominence due to their potential to circumvent the side effects associated with synthetic medications [15]. This study investigated the antioxidant properties and sedative characteristics of selected plants, positioning them as potential promoters of brain health in the context of sleep disorders.

The analysis of the samples revealed that *Cnestis ferruginea* had a greater antioxidant potential than *Piper guineense*. The animal experiments focused on the samples of *Cnestis ferruginea*. The sedative effects were evaluated in male Wistar rats subjected to chronic stress. The study examined the time it took for the rats to fall asleep and the total duration of sleep in different groups, including a control group, a stress group, a stress group treated with diazepam (as a positive control), and a stress group treated with two different doses of the plant sample to explore its potential anxiolytic effects. The results indicated that chronic stress reduced the duration of sleep in rats, but treatment with diazepam increased the amount of time spent sleeping, indicating a sedative effect. Similarly, treatment with the plant sample for its potential antidepressant effects increased the duration of sleep in stressed rats. Both doses of the plant sample significantly prolonged the duration of sleep, suggesting a sedative effect. Further analysis revealed that the stress-induced group exhibited a longer period of immobility in the forced swim test, indicating a higher level of despair compared to the control group. Administration of both doses of *Cnestis ferruginea* ethanolic extract significantly reduced the duration of immobility in the rats, demonstrating a potential for alleviating depressive symptoms [16]. Additionally, the study observed improvements in acetylcholinesterase levels in the brain after treatment with *Cnestis ferruginea*, indicating a potential mechanism for relieving depressive symptoms.

5. Conclusion

In concluding our investigation, our examination into the bioactive constituents of *Cnestis ferruginea* and *Piper guineense* indicates their potential as agents that elevate mood. The combination of flavonoids, phenolics, saponins, and tannins present in both plants, as well as the identification of terpenoids in *Cnestis ferruginea*, lays the foundation for considering their role in supporting mental well-being.

Given the contemporary interest in employing food as a therapeutic intervention for mental health, our research closely examines the anxiolytic and sedative properties of these botanical species. Their nutrient-rich profiles suggest that they may play a significant role in maintaining optimal brain function, particularly in relation to sleep disturbances.

As individuals increasingly seek treatments with minimal side effects, our emphasis on the anxiolytic and sedative properties of these plants aligns with the evolving trends in mental healthcare. Notably, *Cnestis ferruginea* stands out due to its remarkable stress-reducing capabilities in comparison to *Piper guineense*, prompting us to explore its potential as a therapeutic agent in greater detail.

Our investigation into the effects of *Cnestis ferruginea* on sleep, particularly in the context of chronic stress, yielded promising outcomes. The observation that stressed rats ex-

hibited prolonged sleep duration after receiving a dose of the plant extract, coupled with its capacity to improve their mood under stressful conditions, suggests that it may serve as a dual-purpose intervention in alleviating stress-related behavioral changes and depressive symptoms.

Conflicts of Interest

The authors declare no conflicts of interest.

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